# CM 22102 - TRIPLE SUGAR IRON AGAR
# (ISO 6785:2001 / ISO 6579:2017)

## INTENDED USE

Foridentificationof gram-negative enteric bacteria on the basis of sugar fermentation & $H_2S$ production.

## PRODUCT SUMMARYANDEXPLANATION

TripleSugarIronAgar was originallyproposed by Sulkin and Willett and modified by Hajna for identifying Enterobacteriaceae. This medium is a modification of the Kligler Agar where sucrose was added to differentiate Proteus and Hafnia (sucrose positive) from Salmonella and Shigella (sucrose negative). It complies with the specifications given by EN ISO 6785, ISO 6579 and APHA.

## COMPOSITION

| Ingredients | Gms / Ltr |
|---|---|
| Agar | 12.000 |
| Lactose | 10.000 |
| Peptic digest of animal tissue | 10.000 |
| Casein enzymatic hydrolysate | 10.000 |
| Sucrose | 10.000 |
| Sodium chloride | 5.000 |
| Yeast extract | 3.000 |
| Beef extract | 3.000 |
| Dextrose | 1.000 |
| Ferrous ammonium citrate | 0.300 |
| Sodium thiosulphate | 0.300 |
| Phenol red | 0.024 |

## PRINCIPLE

The Medium is composed of Peptic digest of animal tissue, Beef extract and Yeast extract which provides nitrogen, carbon and vitamins required for bacterial growth. Triple Sugar Iron Agar consists of three carbohydrates; Dextrose, Lactose and Sucrose. When carbohydrates are fermented, acid production is detected by Phenol red pH indicator. Sodium thiosulphate is reduced toHydrogen sulphide and Hydrogen sulphide reacts with iron salt yielding typical black iron sulphide. Ferrous ammonium citrate is a Hydrogen sulphide ($H_2S$) indicator and gives a typical black precipitate. Sodium chloride maintains osmoticbalance of the medium. Agar is used as a solidifying agent.

## INSTRUCTION FOR USE

Dissolve 64.62 grams in 1000ml distilled water.
Gently heat to boiling with swirling to dissolve the medium completely.
Dispense into tubes as desired.
Sterilize by autoclaving at 15 psi (121°C) for 15 minutes
Allow the medium to set in sloped form with a butt of depth about 2.5cm-5cm.

Note: For better results, the medium can be sterilized by autoclaving at 10 psi pressure (115°C) for 15 minutes

## QUALITY CONTROL SPECIFICATIONS

| | | |
|---|---|---|
| Appearance of Dehydrated powder | : | Light yellow to pink, homogeneous free flowing powder |
| Appearance of Prepared medium | : | Pinkish red coloured, clear to slightly opalescent gel |
| pH (at 25°C) | : | 7.4± 0.2 |

## INTERPRETATION

Culturalcharacteristics observed after an incubation.

| Microorganism | ATCC | Inoculum (CFU/ml) | Growth | Slant | Butt | Gas | $H_2S$ | Incub* Temp | Incub* Period |
|---|---|---|---|---|---|---|---|---|---|
| Citrobacter freundii | 8090 | 50-100 | Luxuriant | Acidic Reaction | Acidic Reaction | Positive Reaction | Blackening of medium | 35-37°C | 18-24 Hours |
| # Klebsiella aerogenes | 13048 | 50-100 | Luxuriant | Acidic Reaction | Acidic Reaction | Positive Reaction | No Blackening of medium | 35-37°C | 18-24 Hours |
| Escherichia coli | 25922 | 50-100 | Luxuriant | Acidic Reaction | Acidic Reaction | Positive Reaction | No Blackening of medium | 35-37°C | 18-24 Hours |
| Klebsiella pneumoniae | 13883 | 50-100 | Luxuriant | Acidic Reaction | Acidic Reaction | Positive Reaction | No Blackening of medium | 35-37°C | 18-24 Hours |
| Proteus vulgaris | 13315 | 50-100 | Luxuriant | Alkaline Reaction | Acidic Reaction | Negative Reaction | Blackening of medium | 35-37°C | 18-24 Hours |
| Salmonella Paratyphi A | 9150 | 50-100 | Luxuriant | Alkaline Reaction | Acidic Reaction | Positive Reaction | No Blackening of medium | 35-37°C | 18-24 Hours |
| Salmonella Typhi | 6539 | 50-100 | Luxuriant | Alkaline Reaction | Acidic Reaction | Negative Reaction | Blackening of medium | 35-37°C | 18-24 Hours |
| Salmonella Typhimurium | 14028 | 50-100 | Luxuriant | Alkaline Reaction | Acidic Reaction | Positive Reaction | Blackening of medium | 35-37°C | 18-24 Hours |
| Shigella flexneri | 12022 | 50-100 | Luxuriant | Alkaline Reaction | Acidic Reaction | Negative Reaction | No Blackening of medium | 35-37°C | 18-24 Hours |
| Escherichia coli | 8739 | 50-100 | Luxuriant | Acidic Reaction | Acidic Reaction | Positive Reaction | No Blackening of medium | 35-37°C | 18-24 Hours |
| Klebsiella pneumoniae | 10031 | 50-100 | Luxuriant | Acidic Reaction | Acidic Reaction | Positive Reaction | No Blackening of medium | 35-37°C | 18-24 Hours |
| Shigella flexneri | 12022 | 50-100 | Luxuriant | Alkaline Reaction | Acidic Reaction | Negative Reaction | No Blackening of medium | 35-37°C | 18-24 Hours |

Acidic Reaction =Yellowing of the medium, Alkaline Reaction =Red colour of the medium
Blackening of the medium = Positive Reaction, No Blackening of the medium = Negative Reaction
Incub*=Incubation
# Formerly known as Enterobacter aerogenes

## PACKAGING

In100&500gm packaging size.

## STORAGE

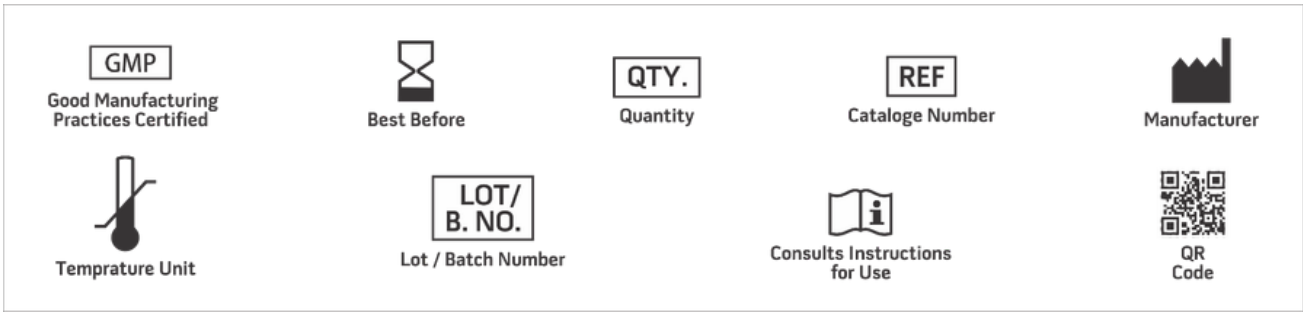
Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers below 25°C and protect from direct Sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.
Product Deterioration: Do not use, if powder show evidence of microbial contamination, discoloration, drying, or other signs of deterioration.

## DISPOSAL

Afteruse,prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

## REFERENCES

1. Russell, F. F. The isolation of typhoid bacilli from urine and feces with the description of a new double sugar tube medium. J. Med. Res. 25:217. (1911.
2. Kligler, I. J. A simple medium for the differentiation of members of the typhoid-paratyphoid group. Am. J. Public Health 7:1042-1044. (1917).
3. Kligler, I. J. Modifications of culture media used in the isolation and differentiation of typhoid, dysentery, and allied bacilli. J. Exp. Med. 28:319-322. (1918).
4. Sulkin, S. E., and J. C. Willett. A triple sugar-ferrous sulphate medium for use in identification of enteric organisms. J. Lab. Clin. Med. 25:649-653. (1940).
5. ISO 6579 Microbiology of food and animal feeding stuffs. Horizontal method for the detection of Salmonella spp Standard Methods for the Examination of Dairy Products. APHA, (1972)




NOTE: Please consult the Material Safety Data Sheet for information regarding hazards and safe handling Practices.

*ForLab Use Only